Characteristics of water relations in seedling of *Machilus yunnanensis* and *Cinnamomum camphora* under soil drought condition

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Abstract: The soil drought stress experiment in different durations (no watering within 3d, 6d, 9d, 11d individually) was conducted to study the drought-resistant capacity of one-year-old seedlings for the native tree species (*Machilus yunnanensis*) in Yunnan Province and the introduced tree species (*Cinnamomum camphora*). The leaf water potential, chlorophyll content, proline content and plasma membrane permeability for two species seedlings were measured in different soil drought conditions. The results showed that, on the 9th day of drought stress, the leaf water potential of two species decreased obviously, whereas the free proline content and plasma membrane permeability increased sharply. On the 11th day, the leaf water potential of *C. camphora* seedlings was lower than that of *M. yunnanensis* seedlings; the plasma membrane permeability in *C. camphora* seedling leaves increased much more than that in *M. yunnanensis* seedling leaves, which showed that the injury to the former by soil drought stress was more severe than that to the latter. The free proline content in *M. yunnanensis* seedling leaves continued to increase on the 11th day, but that in the *C. camphora* seedling leaves started to drop obviously, indicating that the reduction of osmotic regulation substance in *C. camphora* seedling leaves after the 11th day was unable to maintain the osmotic balance between the plasma system and its surroundings and the water loss occurred inevitably. Comprehensively, *M. yunnanensis* seedlings enhanced the drought-resistance in the course of soil drought stress by maintaining higher leaf water potential and by increasing osmotic regulation substance to promote cell plasma concentration and maintain membrane structure integrity so as to reduce water loss. The subordination function index evaluated with fuzzy mathematic theory also showed that the drought-resistant capacity of *M. yunnanensis* seedlings was stronger than that of *C. camphora* seedlings.

Keywords: Soil drought; Water potential; Plasma membrane permeability; *Machilus yunnanensis*; *Cinnamomum camphora*

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Introduction

Soil drought not only influences agriculture and forestry production, but also brings much trouble for cultivating plants of gardening in the urban. Although the mean annual precipitation of Kunming City, China is 1079 mm, its distribution is quite uneven in different seasons. The precipitation in dry season from November of each year to April of the next year only accounts for about 11% of the total amount of the year. Therefore, water affecting the growth of plants always becomes the limiting factor to plant growth in the dry season in Kunming. Irrigation is generally the most costly measure in the tending measures for local gardening and urban forestation. The key approach is reasonably to select tree species for promoting economical, healthy and sustainable development of landscaping construction in Kunming. The native tree species that have relatively high drought resistant, cold resistant and air pollution tolerant capacities should be planted widely. *Machilus yunnanensis* is one of the most potential native ornamental tree species of Yunnan Province with local characteristics, while *Cinnamomum camphora* is one of the most common exotic tree species, commonly planted in Kunming in recent years., In the present study, the leaf water potential, chlorophyll content, proline content and plasma membrane permeability for the two species seedlings were measured in different soil drought conditions in order to compare the drought resistance of two tree species and to find out which species would be more adaptable to be planted in the dry season in Kunming. The results may provide some theoretical bases for ornamental tree species selection in Kunming City.

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Materials and methods

Sixty one-yr.-old seedlings in similar size for each of the two tree species, *M. yunnanensis* and *C. camphora*, were transplanted into plastic nutrition bags with 18 cm×15 cm×18 cm in volume on Feb. 20th, 2006. The planting medium was composed of the local red soil in Yunnan, humus soil and pearlite, and the mixed proportion of the 3 components was 4:3:1, with pH 6.0. And 10 g of NPK compound fertilizer were added to each bag.

The experiment was carried out on the 7th floor of the Building in Southwest Forestry College, with regular sunshine but no risk to be watered by rainfall. After 40 days of normal watering and tending (1 L of water was used for each bag every two days) , the water control experiment started on April 3rd, 2006. All seedling bags were fully watered, and then the following 5 treatments of water control were applied: first is no watering within 3d; second no watering within 6d; third no watering within 9d; fourth no watering within 11d; fifth CK-with normal watering (one irrigation with 1L of water for each bag every two days). The physiological indices were measured next morning for each treatment.

Measurement of physiological indices

Chlorophyll content in leaves was extracted by mixed solution of 2 units of acetone and 1 unit of ethyl with extraction method for 24 h (Yang 2002). Free proline content in the leaves was extracted by sulpha-salicylic acid and measured by triketone colorimetric analysis method (Hou 2004). Plasma membrane

permeability was measured by relative conductivity method (Hou 2004). The injury of plasma membrane was calculated by comparing with the CK group. The formula is as follows:

$$Pi(\%) = \frac{(Pt - Pck)}{(1 - Pck)} x100\ \% \quad (1)$$

where, *P*i is the injury degree of plasma membrane, *P*t the plasma membrane permeability at different drought stress levels, and *P*ck is the leaf permeability of CK group.

Leaf and soil water potential were measured with HR-33-TR Microvoltmeter produced by WESCOR Co. Ltd., USA. The balance time is 0.5h for leaf samples and 12 h for the soil.

Results

The change of leaf water potential

Leaf water potential is a direct index expressing water shortage and moisture content inside the plants. It was reported that there was good linear relationship between the predawn leaf water potential and the soil water potential (Fu *et al*. 2005). The predawn leaf water potential is usually lower than the soil water potential when there is enough moisture in the soil. As the drought in the soil intensifies, the predawn leaf water potential is higher than the soil water potential, and then the plant will die because of being unable to absorb water from the soil and wilt for losing water.

The leaf water potential dropped along with the decline of soil water potential (Fig. 1). In the early stage of drought stress (3d, 6d), the soil water potential was higher than the leaf water potential, showing that no drought stress occurs at this stage. However, after the 6th day, the soil water potential was about equal to that of leaves of two species seedlings. After the 9th day of drought stress, the leaf water potential of *C. camphora* seedlings dropped more rapidly than that of *M. yunnanensis* seedlings, indicating that the drought stress damage to *C. camphora* seedlings happened earlier than that to *M. yunnanensis* seedlings. It was reported that the activity of PS II would be weakened and depleted when the leaf water potential dropped to -2.5Mpa (Pang 1999). Fig. 1 showed that the leaf water potential of *C. camphora* seedlings dropped to -3.845Mpa on the 9th day, much lower than -2.5Mpa, which means that PS II of *C. camphora* seedlings had already been damaged.

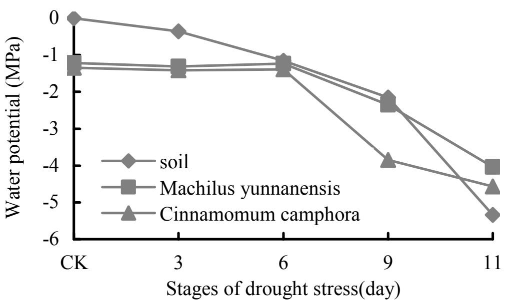

Fig.1 Change of seedling leaf water potential of *Machilus yunnanensis* and *Cinnamomum camphora* and soil water potential

The change of chlorophyll content

The decline of chlorophyll content is one of the major indexes showing leaf senescence (Thimann 1980). From Fig. 2, there was a difference in chlorophyll contents in seedling leaves of two species during the periods of drought stress. At the initial period of the experiment (3d, 6d), there is no evident change in chlorophyll contents for two species. The chlorophyll contents of *M. yunnanensis* increased a little on the 9th day of drought stress, which might be the active adaptability to the drought environment in order to maintain the normal photosynthesis. Meanwhile, the chlorophyll contents of *C. camphora* dropped obviously. On the 11th day, the chlorophyll contents of *C.camphora* raised abruptly. This might result in the increment of the unit quantity of leaf chlorophyll contents when its leaf water content dropped obviously, and it might be not caused by active adaptation by the seedlings. The chlorophyll contents of *M. yunnanensis* raised little on the 11th day, and it indicated that *M. yunnanensis* can keep its normal chlorophyll contents to photosynthesis. Along with the increase of soil drought stress, however, the cell membrane started to be injured because of water loss, and the plasma would leak out, the normal physiological biochemistry pathways would be blocked, and the chlorophyll contents would drop eventually.

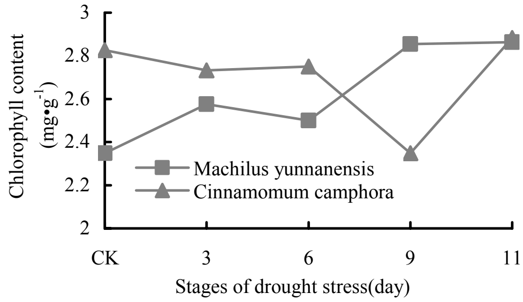

Fig.2 Change of chlorophyll contents in seedling leaves of *Machilus yunnanensis* and *Cinnamomum camphora*

The change of free proline content

Free proline is one of the components of plant protein, which exists widely in plants at free status. Under the adverse circumstance conditions (such as drought, salinity, frost, etc.), the free proline content inside the plant can increase by 10-100 times, and may account for over 40% in the total quantity of amino acids. Free proline often accumulates to a very high quantity, particularly under drought stress situation (Yan *et al*. 2000). It has been indicated by many researches that the increment of free proline content is a sort of adaptation to drought stress.

On the 3rd and 6th day, there was no evident change in free proline content in the seedling leaves for two species in comparison with the CK groups (Fig. 3). While on the 9th day of drought stress, the free proline content in seedling leaves of two species increased sharply. By the 11th day, the free proline content in seedling leaves of *M. yunnanensis* was still increasing, was 30.5 times of the CK group, but that in seedling leaves of *C. camphora* started to drop, which was only 11.6 times of the CK group at the respective time.

The change of plasma membrane permeability and injury of plasma membrane

Cell membrane is the major passage for substance exchange between the cells and the environment, which plays a very important role in maintaining the microenvironment and keeping normal metabolism inside the cells. The relative conductivity of cell membrane may reflect the membrane stability under the adverse circumstances. The plasma membrane permeability will

increase because of the leakage of salts and organic substances from inside of the cells when the permeability of cell membrane increases because of the damage to its structure and function under varied adverse circumstances. The injured degree of plasma membrane and the resistant capacity of the studied species to adverse environment can be reflected by determining the variation of conductivity of the extract solution. The heavier the cell membrane is injured, the more ions leak out and the greater of the permeability is.

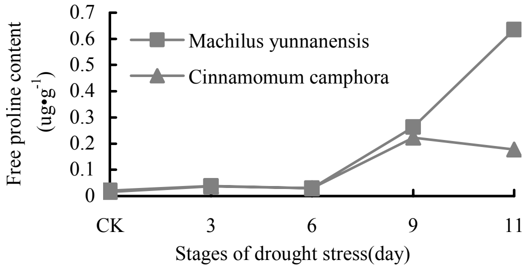

Fig.3 Change of the free praline content in seedling leaves of *Machilus yunnanensis* and *Cinnamomum camphora*

On the 3rd and 6th day, there was no obvious change in plasma membrane permeability of two species, indicating that the growing of both species were not yet influenced by soil drought stress at the early stages (Table 1). On the 9th day, the permeability of seedling plasma membrane of two species increased, showing that the seedlings of two species were somewhat impacted by drought stress at this stage, but the cell membrane conductivity of *C. camphora* increased more than that of *M. yunnanensis*. On the 11th day, the plasma membrane permeability of *Cinnamomum camphora* seedlings was 34.94%, which was 4.07 times as high as that of the CK group; while the plasma membrane permeability of *M. yunnanensis* seedlings was 21.17%, only 1.3 times that of its CK group. These data indicated that the resistant capacity to dehydration of *M. yunnanensis* seedling leaves is stronger than that of *C. camphora.*

Table 1. Variation of permeability and injury of plasma membrane at different stages of drought stress

Stages of drought stress	Plasma membrane permeability (%)		Injured degree of plasma membrane (%)	
	Machilus yunnanensis	*Cinnamomum camphora*	*Machilus yunnanensis*	*Cinnamomum camphora*
CK	15.80	8.58	-	-
3d	16.53	11.23	0.87	2.90
6d	18.34	16.24	3.02	8.38
9d	19.07	28.20	3.89	21.46
11d	21.17	34.94	6.38	28.83

Variances analysis of the physiological indices at different stages of soil drought stress

Each physiological index of two species was analyzed at different stages of soil drought with SPSS software. It was shown by the LSD multiple comparison that there was significant difference in plasma membrane permeability for two species at different stages of drought stress ($p<0.05$), there were no significant differences in leaf water potential and free proline content on the 3rd and 6th day individually, while on the 9th and 11th day, the significant differences were shown respectively for two species (Table 2, 3).

Table 2. Variances analysis of each physiological index of *Machilus yunnanensis* seedlings at different stages of drought stress

Indices measured	Different stages of drought stress			
	I	II	III	IV
Leaf water potential (Mpa)	1.31±0.095a	1.23±0.022a	2.35±0.042b	4.03±0.06c
Chlorophyll contents (mg/g)	2.58±0.055a	2.50±0.144a	2.85±0.072b	2.86±0.163b
Free proline content (μg/g)	0.04±0.002a	0.03±0.001a	0.26±0.014b	0.64±0.045c
Membrane permeability (%)	16.5±0.008a	18.3±0.002b	19.07±0.004c	21.17±0.006d

Notes: Mean±S.D, Mean is the average of 3 duplicates, there are no significant differences among data with the same letter attached (a=0.05). I : no watering within 3d; II : no watering within 6d; III: no watering within 9d; IV: no watering within 11d.

Table 3. Variances analysis on each physiological index of *Cinnamomum camphora* seedlings at different stages of drought stress

Indices measured	Different stages of drought stress			
	I	II	III	IV
Leaf water potential(-Mpa)	1.42±0.062a	1.39±0.046a	3.85±0.141b	4.28±0.104c
Chlorophyll contents(mg/g)	2.73±0.08a	2.75±0.09a	2.35±0.04b	2.88±0.07a
Free proline content(μg/g)	0.04±0.003a	0.03±0.026a	0.22±0.043b	0.18±0.005c
Membrane permeability (%)	11.23±0.003a	16.24±0.003b	28.2±0.001c	34.95±0.028d

Notes: Mean±S.D, Mean is the average of 3 duplicates, there are no significant differences among data with the same letter attached (a=0.05) . I : no watering within 3d; II : no watering within 6d; III: no watering within 9d; IV: no watering within 11d.

Evaluation on drought-resistant capacity of seedlings of two tree species

The drought-resistant capacity is an integrated characteristic of plants under soil drought stress, it would be very hard to evaluate accurately the drought-resistant capacity of a plant species by the individual indices. The results indicated that each physiological index in different stages of drought stress was not always consistent with one another; this meant that the way of plants responding to drought stress is varied. In other words, there were multiple approaches to respond to drought stress by different plant species. The drought-resistant capacity of seedlings of two species was evaluated by the subordinate function method (Tao 1982; Gong 1989), the formulae are as follows:

(1)If there is a positive correlation between the index and the drought-resistant capacity, there is:

$$X_{(u)}=(X-X_{min})/(X_{max}-X_{min}) \quad (1)$$

(2)If there is negative correlation between the index and the

drought-resistant capacity, there is:

$$X_{(u)}=1-(X-X_{min})/(X_{max}-X_{min}) \quad (2)$$

where, $X_{(u)}$ is the subordinate function values, X the mean value of each index, X_{max} the maximum value of each index, and X_{min} the minimum value of the index. The drought-resistant capacity was evaluated by the mean sum value of the subordinate function values of all indices for each studied tree species. The more the mean value of the subordinate function values was, the stronger the drought-resistant capacity of the tree species was (Table 4).

Table 4. Subordinate function values of seedlings of the two studied tree species

Tree species	Index						
	Leaf water potential	Chlorophyll content	Free proline content	Plasma membrane permeability	Injury of plasmamembrane	Mean	Order
Machilus yunnanensis	0.71	0.46	0.29	0.56	0.52	0.51	1
Cinnamomum camphora	0.62	0.32	0.39	0.57	0.52	0.49	2

The results indicated that the drought-resistant capacity of *M. yunnanensis* seedlings was stronger than that of *C.camphora*. The young leaves of *M. yunnanensis* seedlings started to show temporary wilting status on the 9th day of drought stress when the soil water potential was -2.16MPa, the adult leaves still maintained the natural state; while the young leaves and the adult leaves of *C. camphora* seedlings were curled and wilted.

Conclusions

The pot culture experiment indicated that there was no obvious change of each physiological index measured between two species on the 3rd and 6th day of drought stress. On the 9th day of drought stress, the leaf water potential of two species decreased obviously, whereas the free proline content and plasma membrane permeability increased sharply, the chlorophyll contents of *M. yunnanensis* raised a little, but that of *C. camphora* dropped obviously. On the 11th day, the leaf water potential of two species continued to drop while the leaf water potential of *C. camphora* seedlings was lower than that of *M. yunnanensis* seedlings. The plasma membrane permeability of two species continued to increase while the plasma membrane permeability in *C. camphora* seedling leaves increased much more than that in *M. yunnanensis* seedling leaves, which showed that the injury to the former by soil drought stress was more severe than that to the latter. The free proline content in *M. yunnanensis* seedling leaves continued to increase on the 11th day, but that in the *C. camphora* seedling leaves started to drop obviously. Along with the increment of drought stress, the leaf water potential of two species continued to drop, the plasma membrane permeability of two species continued to increase and each physiological index of *C. camphora* changed more than that of *M. yunnanensis*.

The drought-resistant capacity of *M. yunnanensis* seedlings was stronger than that of *C. camphora* under the same drought intensity. Namely, *M. yunnanensis* seedlings had stronger capacity of maintaining the integrity of plasma membrane structure by keeping higher water potential and increasing osmotic regulation substances during the process of drought stress to reduce water loss. This fact was also well proven by the comprehensive evaluation with subordinate function method.

Discussion

Though the change tendency of water related physiological indices of two species were basically similar, the adaptive responses by seedlings of two species to different soil drought stress were different. However, the comparative study on soil drought stress to *M. yunnanensis* and *C. camphora* was only done with pot experiment for one-year-old seedlings, further study on the adult plants grown in the practical urban environment should be done to verify the results.

The stronger drought-resistant capacity of *M. yunnanensis* seedlings showed that seedlings of the native tree species could better adapt to the dry season in Kunming. And there should be some correlations with the drought resistance between the seedlings and the adult plants. It is a quite common phenomenon nowadays in urban area to plant many exotic species with huge cost for emphasizing too much on superficial appearance in landscape planning and design. The native plant species with superior characteristics such as stronger resistance to varied adverse conditions, wide adaptability, healthy growth may produce higher comprehensive social, ecological and economic benefits.

The climate in Kunming is characterized by distinct alternation of dry season with rainy season. Since there is little rainfall in the spring when most of tree species sprout and start fast growing, the plants are more sensible to soil drought stress. Apart from normal tending and management, appropriate irrigation is critical to guarantee normal growth and development of the trees in the dry season. It is of great importance to make full use of the native species such as *M. yunnanensis* in urban forest construction to reduce water resource consumption, to lower tending cost and to show local specialty.

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